INTRODUCTION
Countries within the tropical belt have a predilection towards multiple pathogens due to the year-round circulation of respiratory pathogens. This study aims to data from the respiratory disease sentinel surveillance programme of the Singapore military in order to compare patients with one (mono-pathogen) and two pathogens detected (dual pathogen) and describe observed difference in clinical characteristics.

METHODS
Study site and population. Sentinel surveillance for febrile respiratory patients were performed at five major sites during a study period from May 2009 to Oct 2012.

Laboratory methods. Nasal wash samples obtained were stored in fridge at 4°C and transported to the laboratory using carriers with ice packs within 24 h. Molecular Diagnostic Testing was done by the extraction of nucleic acids using the DNA mini kit (Qiagen, Inc, Valencia, CA, USA) which was then tested using multiplex PCR assays coupled with bead array detection technology (Replex I and II, version 2.0, Qiagen, Inc, Valencia, CA, USA) which can simultaneously detect and subtype 18 different pathogens.

Statistical methods.
- Demographic characteristics for controls, mono-pathogens, dual pathogens and patients with more than two pathogens were analysed and compared using descriptive statistics.
- Analysis on the prevalence of co-existing pathogens. Interval/ratio variables were compared using one-way ANOVA.
- Comparison of nominal variables with expected frequencies ≤5 with Fisher’s exact test, expected frequencies >5 was done using Pearson’s chi-square test.
- Analysis focused on comparing patients with one and two pathogens, a total of 11 mono-pathogens and 18 dual-pathogen pairs were available for comparison.
- Permutation tests were conducted to compare the number of symptoms observed between mono-pathogen and dual-pathogen patients for each pathogen as a proxy for severity of infection.
- Assess differences in symptom expression by comparing dual pathogens against mono-pathogens for mean proportions of 16 symptoms (or signs). Empirical proportions of symptoms with 95% confidence intervals (CIs) for both mono-pathogens and dual-pathogen were calculated and compared using Pearson’s chi-square test at a significance level of 0.05.
- Dual infections with statistically different results from their respective viral respiratory mono-infections were highlighted.
- R Statistical Software (version 3.0.3) was used to perform all statistical analyses.

RESULTS
Of 7733 samples of patients tested, 45.8% had mono-pathogens and 20.2% had dual pathogens detected. No pathogens were picked up in 30.9% samples, while 3.1% samples had more than two pathogens.

Among dual pathogens, virus-bacterial pairs were the most common at 76.0%, followed by bacteria-bacteria (15.2%) and virus-virus pairs (8.8%).

Demographics for patients and controls are detailed in Table 1.

Figure 1 details proportions of mono-, dual and more than 2 pathogens detected for each pathogen.

Influenza A (80.0%), Influenza B (73.0%) and Mycoplasma (70.6%) were most commonly associated with mono-infections while Adenovirus was most commonly associated with dual-infections (62.9%).

Figure 2 depicts the incidence of dual-pathogen pairs
Among dual pathogens, there were 13 virus-bacteria and 5 virus-virus combinations with more than 15 observations each.

The most common virus-virus pair was that of influenza A with enterovirus;
Of bacteria-virus interaction, Haemophilus influenzae with S. pneumoniae was most common.

CONCLUSION
We have described the aetiology of dual pathogens causing FRI in the tropical setting and compared differences with mono-pathogens with regard to observed clinical manifestations.

The presence of higher incidences of certain symptoms with specific pathogen pairs is indicative of underlying complex microbial interactions and affirms existing microbiological co-pathogen studies.

However, many of these processes are still not well explored in existing literature, opening many opportunities for further research into this area.